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Analysis of Aristolochia Longa L. Medicinal Plant from Algeria

Zohra Lamari and Houria Negache

Abstract

In recent time, the therapeutic use of medicinal plants has increased all over the world. The efficacy of herbs for curative purposes is often accounted of its mineral and organic constituents. Neutron activation analysis (INAA) has been applied to mineral determination of *Aristolochia Longa* (bereztem), medicinal plant used to cure some diseases observed in Algeria especially cancer. In this work the mass fractions of Cr ($15.22 \pm 3.5 \mu\text{g/g}$), Na ($269.98 \pm 25.01 \mu\text{g/g}$), La ($0.478 \pm 0.041 \mu\text{g/g}$), K ($1.33 \pm 0.23 \mu\text{g/g}$), Br ($1.2 \pm 0.19 \mu\text{g/g}$), As (0.697 ± 0.038) and Sb ($66.09 \pm 11.24 \mu\text{g/g}$), were determined. This herb was collected from Taourirt Aden Berber village situated in Northern Algeria. Five elements were quantified in certified AIEA standards IAEA-V10 and IAEA-SL1 for checking the accuracy of our procedure. It was noteworthy the values obtained from this work are in good agreement with the certified values, the Z-score values for all elements were $|Z| < 3$. We believe that herb is natural and harmless compared with chemical drugs. Unfortunately the potential toxicity due to the Aristolochia Acids content has required the analysis of *Aristolochia Longa* by CG/MS and HPLC to highlight this compound. The standard of Aristolochic Acid (Sigma A5512-25 mg Yellow powder lot # wxbb6331V^{PCODE}) was used as reference.

Keywords: *Aristolochia Longa*, INAA, organic compounds, public health, Algeria

1. Introduction

Bereztem is the common name of *Aristolochia Longa*, this herb with a delicate aromatic odor is wrongly used as medicinal plant in Algeria [1, 2] and other countries. We find these plants in the temperate and tropical regions [3]. Since antiquity various *Aristolochia* and *Asarum* species have been used in herbal medicines in obstetrics and in treatment of Intestinal affections, coetaneous diseases, wounds, heart palpitation or snakebite, festering wounds, and tumors [4, 5], indeed 60% of drugs approved for cancer treatment are of natural origin [6, 7]. B. Benarba report the cytotoxic and apoptogenic activities of an aqueous extract of *A. longa* in the Burkitt's lymphoma BL41 cell line [8]. Considered also as antidote against some poisonings [9]. It was reported [10, 12] that plants remain in use today especially in the Chinese medicine. All parts of the plant are used in herbal preparations, and aristolochic acids are present in the roots, stems, leaves, and fruit [11, 12]. Exposure to this acid could potentially occur through the ingestion or skin contact to treat wounds, note no published studies of skin absorption of aristolochic acids in humans or experimental animals were found. However certain plant as canadense

leaves cause the dermatitis [13]. The dried rhizome of *Aristolochia Longa* is the one of the part frequently used often without other ingredients. Several reports indicate the use of complementary and alternative medicine (CAM) and a lot of people in Algeria believe that herb is natural and harmless compared with chemical drugs. Unfortunately, they are unaware of its adverse biological effects. The Aristolochic Acids content, alkaloid components are known to be mutagenic and carcinogenic [14]. The clinical syndrome to the Chinese plants Nephropathy (NCP) was reported in first in Belgium for the women having followed a Chinese slimming diet in 1992 after consumption of herbal weight loss preparations containing *Aristolochia Fangehi* by inadvertence instead *Stephania Tetrandra* [15]. Kupchan and Dосkovitch 1962 [16], have tested the antitumor effects of aristolochic acids in mice and in clinical trials. But when Jackson et al. [17] showed the nephrotoxicity of aristolochic acid the trials were discontinued. Mix et al. [18]; Kumar et al. [19] Described twelve Aristolochic Acid analogues, the major compounds of AA_s include AAI and its demethoxylates derivative, AAII, generally the levels of AAI are higher than AAII, the **Figure 1** shown their structures. The metabolites are excreted in the urine and the feces. Reported half-lives in New Zealand White rabbits for aristolochic acids I and II were 0.12 hours and 0.27 hours, respectively. Studies in rats show that the metabolites of aristolochic acid I are excreted within 24 hours, whereas metabolites of aristolochic acid II are still present in the urine at 72 hours. Furthermore the curative properties of this plant are based only on traditional knowledge and in our country there are no procedures and regulations applicable about the use and marketing to the healing plants. Regrettably *Aristolochia Longa* is easily obtained from local markets. Unlike this herb is forbidden in several countries USA [20], Canada, Taiwan, France, and Belgium. The European Commission (EC) (2000) has prohibited aristolochic acid and its salts, as well as *Aristolochia* species, and their preparations in cosmetic products. The trace element present in *Aristolochia longa* L. rhizomes; determined by Neutron activation analysis (INAA) can may be explain some therapeutic activities. At the same time two (02) methods have been used for the identification of aristolochic acids (AA_s). We made Gas chromatography – Mass spectrometry (CG/MS) and High performance Liquid chromatography (HPLC). This work can constitute a position paper to better use of this natural product by the cancer patients who take this herb.

AAI (AAI) and II (AAII) (EMEA 2000).

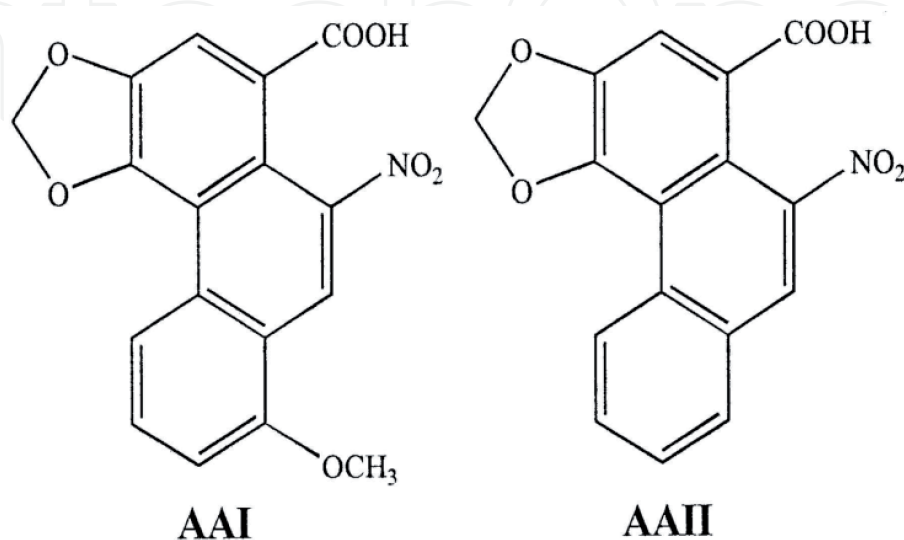


Figure 1.
Chemical structure of nitrophenanthrene carboxylic acids.

2. Experimental

In order to analyze *Aristolochia longa* L., Rhizomes were collected in May 2016 from Taourirt Aden, Algerian village situated in Kabylia region at 120 km south of Algiers city, Algeria. The vegetal material was washed extensively in distilled water as to remove superficial dust. And then dried at room temperature for one week-end. The dry and hard form of these roots was ground in an electric laboratory blender. One part of the fine powder obtained was prepared for neutron Activation Analysis. The triplicate samples were packed in polyethylene thin target and irradiated during four hours (04) in NUR Algerian reactor, 1 MW research reactor. After 04 and 08 days of delay time the acquisitions were done for determination of Cr, Na, La, K, Br, As and Sb. The analysis was done using HpGe – Canberra detector and the elemental concentrations were performed by Calcon software. The count time was twenty (20) hours for medicinal plants and about five (05) hours for the standards. The certified reference material SDM-2TM Lake (sediment marine lyophilized) was used for calibration. The quality control was done using IAEA biological material V10 (hay powder) and AIEA non biological material SL-1 (lake sediment). Another part of this powder herb was used for the identification of Aristolochic Acids by CG/MS; and HPLC techniques. Many extracted methods have been reported in literature [21]. The extract of AA was prepared by adding 10 ml of methanol to 10 g of *A. longa* dry rhizomes powder for the first extraction and 20 ml of light petroleum for the second extraction, solvent extraction the most commonly used for extraction of AAs [22]. After 24 h of maceration under magnetic stirring at room temperature, the mixture was centrifuged, filtered and then concentrated in a rotary vacuum evaporator (The number of siphon age is ten) indeed the quantification of AA in extract products is less complicated as compared to herbal preparations. However to avoid the loss of chemical information's the extraction method should be no selective to explain the therapeutic aspect. The extracted material was analyzed by CG/MS and HPLC in order to detect the potential presence of AAs in *Aristolochia Longa*. The standard of Aristolochic Acid recently acquired (Sigma A5512–25 mg Yellow powder lot # wxbb6331V^{PCODE}.) was used as reference. It should be noted that the Aristolochic acids compounds are produced commercially only as reference standards and as research chemicals [12, 23].

3. Results and discussion

Table 1 show the Algerian medicinal plant studied with the botanical name, common name and the part used for treatment. Five elements were quantified in certified AIEA standards IAEA-V10 and IAEA-SL1 for checking the accuracy of our procedure. The values obtained were showed in **Table 2** with the reported certified values. It was noteworthy the values obtained from this work are in good agreement with the certified values; The **Figure 2** presents graphically the plot of Z-score for our elements.

Common name	Botanical name	Family	Part used for treatments
Berztem	Aristoloshia Longa	Aristolochiaceae	Root, aerial part

Table 1.
Botanical name and parts used of the medicinal plant studied.

Element	IAEA-V10		IAEA-SL1	
	Measured	Reported	Measured	Reported
K [*]	24.65 ± 2.96	21 ± 2		
Cr	6.42 ± 0.798	6.5 ± 0.75	94.92 ± 8.54	104 ± 9
Na [*]	0.692 ± 0.064	0.5 ± 0.3		
Br	10.82 ± 1.73	8 ± 2	6.82 ± 1.73	9.79 ± 1.58
Sb				

^{*}Values given in mg/g.

Table 2.
Quality Control assessment results (µg/g) for the AIEA - certified reference material Samples.

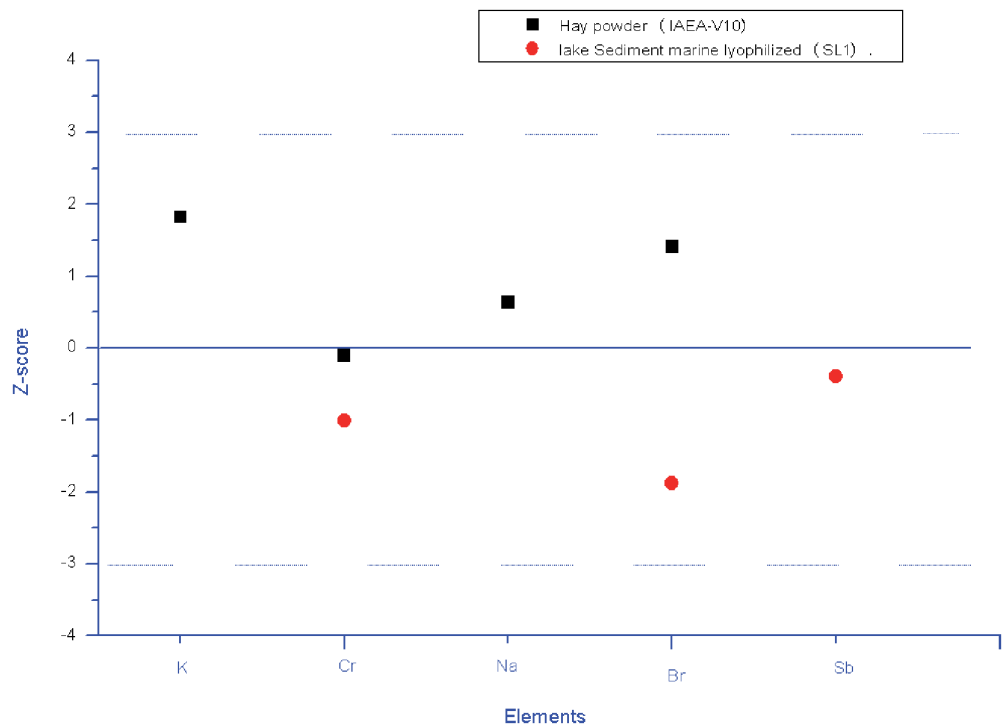


Figure 2.
Z-score values for the elements determined in IAEA standards V10 and SL1.

Table 3 report the elemental concentrations obtained from this work and shows the usefulness of using INAA for the elemental determination, the La, K, Br, As are present at trace levels and the Cr, Sb was found at minor level and Na at the major level. Owing to its high toxicity, the identification of AAs is obviously important indeed these acids cause Aristolochic Acids Nephropathy (NAA), the chronic renal failure according to Grollman et al. [24] and urothelial carcinomas [25, 26].

A new American study reveals that these AAs are more carcinogen than the tobacco [27]. The toxicity of the aristolochic acids has been studied and reported by Mengs and Stotzem [28]. When The Aristolochic Acids extracted from a medicinal plant are traditionally used in China to cure some diseases, the arthritis and the other inflammations. It has been shown by various authors that these Aristolochic acids have directly toxic on the human gene TP53 (gene suppressor of cancer) [29]. No mutations were identified in rats with chronic renal failure not exposed to aristolochic acids. Similar findings have been reported in humans [30]. The complexity of herbal nomenclature systems used in traditional Chinese medicines may have contributed to the potential exposure to aristolochic acids. As well as the

similarity of the Chinese names for *Aristolochia* species and other innocuous herbs can increase risk of inadvertent exposures to aristolochic acids [31].

Even more similar Japanese and Chinese names refer to different plants in Japan and China [32], explained the outbreak of Chinese herb nephropathy in Japan by the substitution of that plant species in Japanese preparations of Chinese herbal medicines. In this study we are analysed the rhizome of *Aristolochia Longa* to identify the aristolochic acid much to our surprise this organic compound is not reveled in our herb. The **Figures 3** and **4** shows the results obtained by CG/MS and HPLC, the characteristic retention times of AAI Acid present in our reference standard are 42.88 min and 21.758 min relative to the analysis by CG/MS and HPLC respectively.

Element	Aristoloshia Longa
Cr	15.22 ± 3.5
Na	269.98 ± 25.01
La	0.478 ± 0.041
K	1.33 ± 0.23
Br	1.2 ± 0.19
As	0.697 ± 0.038
Sb	66.09 ± 11.24

Table 3.
Elemental Concentrations (µg/g) for Aristoloshia Longa rhizome.

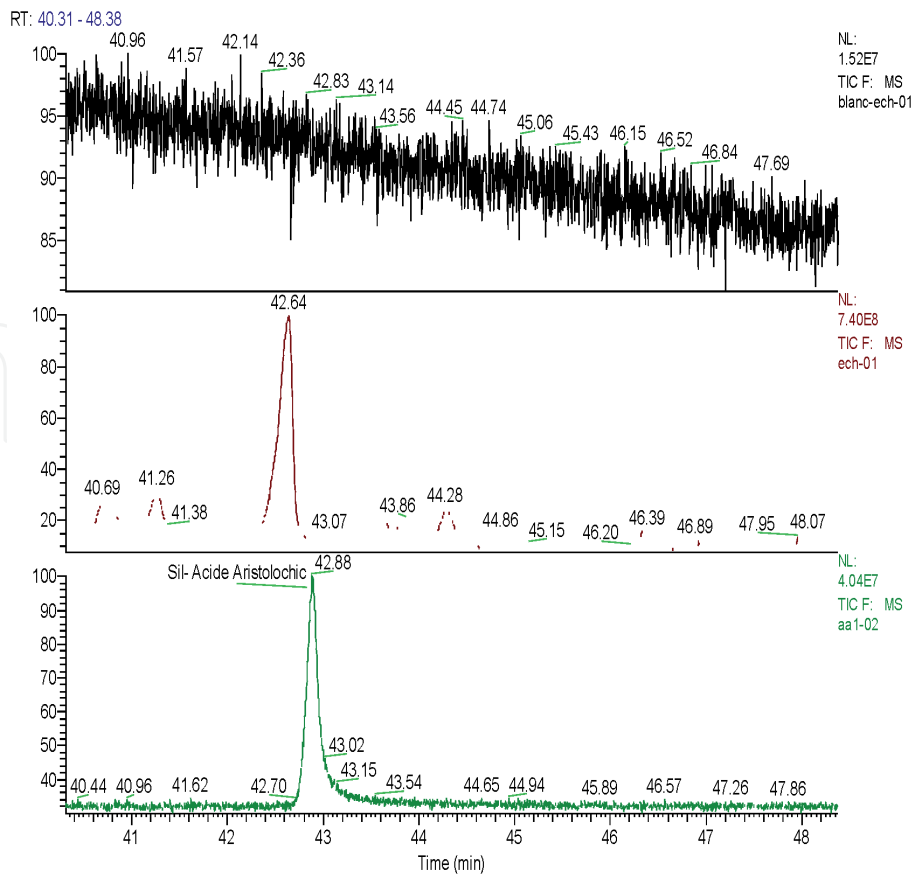


Figure 3.
CG/MS spectrum analysis of our herb (rhizome) and (AAI) standard. (Sigma A5512–25 mg Yellow powder lot # wxbb6331V^{PCODE})

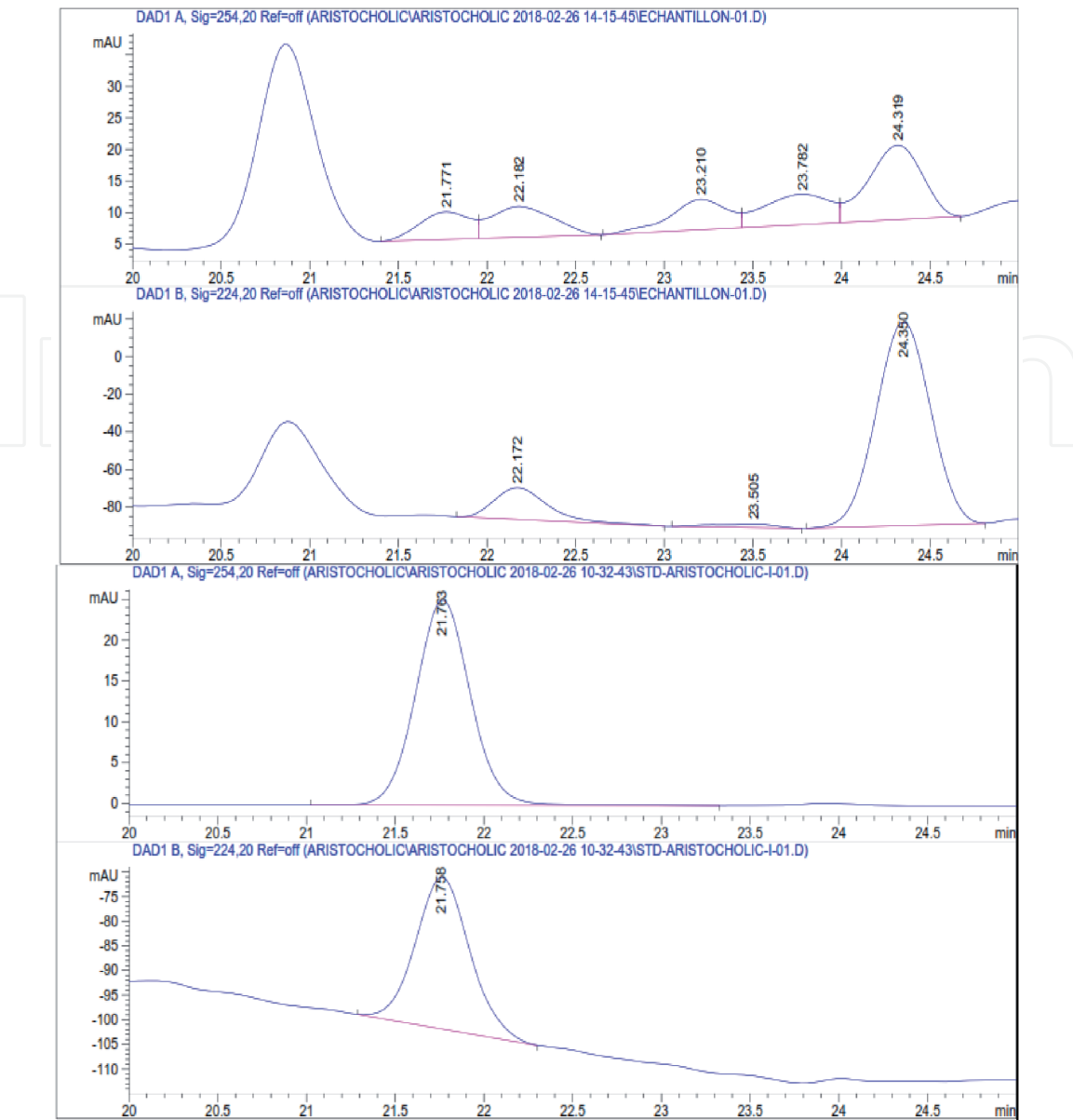


Figure 4. HPLC spectrum analysis of bereztem roots and (AAI) standard. (Sigma A5512–25 mg Yellow powder lot # wxbb6331V^{PCODE})

The Aristolochic acids content of plants varies depending for the same plant species, where it was grown, the time of year, and other factors, that might explain our results? The sample matrix components can influence the limits of detection for Aristolochic acids. We suggested also the insufficient amount of our plant we used 10 mg fine powder of bereztem roots. Although the protocol experimental described by Cherif H.S., and all has been reproduced, in our work, these authors identified the Aristolochic acid (AAI) in the rhizome of bereztem collected in the other city of Algeria: Blida (small city) located about 47 km in southwest of Algiers. While our plant is collected in the region of Taourirt Aden in (Kabylia). Broad range of biological activity of AAs, beneficial as well as adverse effects was reported by Kupchan and Doskotch [33]; therefore it would be interesting to determine the organic composition, it is known that the essential oils contained in the rhizome and aerial part of this herb are mainly responsible for the antimicrobial and cytotoxic effect [34]. And then instead to banned the use of herbal remedy containing acid aristolochic it will be possible to separate the useful from the toxic fractions of plant.

4. Conclusion

Seven elements have been determined in *Aristolochia Longa*. L using INAA, technique usually quite for herbs analyses. This plant can be considered as source of trace elements for people who use it. However the traditional healers recommended the use it with care and always for short treatment periods [35]. It is clear that further experiences are planned to confirm or refute our results obtained by CG/MS and HPLC for the identification of AAs. In its warning, the FDA recommended that all botanical remedies known or suspected of containing Aristolochic acids be discarded. People should be largely aware of the regulated of some botanical products as dietary supplements by the FDA under the Dietary Supplement Health and Education Act (DSHEA) of 1994 (FDA 1995) [36].

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Abbreviations

INAA	Instrumental Neutron activation analysis
CAM	Complementary and Alternative Medicine
NCP	Clinical Syndrome to the Chinese plants Nephropathy
NAA	Aristolochic Acids Nephropathy
AAs	Aristolochic Acids
AAI	Acide Aristolochique I
AAII	Acide Aristolochique (demethoxylates derivative)
EC	European Commission
CG/MS	Gas chromatography – Mass spectrometry
HPLC	High performance Liquid chromatography
IARC	International Agency for Research on Cancer
EMA	European Medicines Agency
INCC	National Institute of Forensic Science and Criminology, Algeria
HpGe-Detector	High Purity Germanium Detector

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